Effects of different sources of *Saccharomyces cerevisiae* biomass on milk production, composition, and aflatoxin M₁ excretion in milk from dairy cows fed aflatoxin B₁

B. L. Gonçalves,* J. L. Gonçalves,† R. E. Rosim,* L. P. Cappato,‡ A. G. Cruz,‡ C. A. F. Oliveira,* and C. H. Corassin*¹

*Department of Food Engineering, College of Animal Science and Food Engineering, and

†Department of Animal Sciences, School of Veterinary Medicine and Animal Sciences, University of São Paulo, Pirassununga, SP, Brazil, 13633-900

‡Federal Institute of Education, Science and Technology of Rio de Janeiro, Department of Food Science, Rio de Janeiro, RJ, Brazil, 20260-100

ABSTRACT

The aim of the present study was to evaluate the effect of different sources of *Saccharomyces cerevisiae* (SC) biomass (20.0 g/d) obtained from sugarcane (cell wall, CW; dried yeast, DY; autolyzed yeast, AY) and the beer industry (partially dehydrated brewery yeast, BY) on milk production, fat and protein percentages, and aflatoxin M₁ (AFM₁) excretion in milk from dairy cows receiving 480 µg aflatoxin B₁ (AFB₁) per day. A completely randomized design was used with 2 lactating cows assigned to each of 10 dietary treatments, as follows: negative controls (no AFB₁ or SC-based biomass), positive controls (AFB₁ alone), DY alone, DY + AFB₁, BY alone, BY + AFB₁, CW alone, CW + AFB₁, AY alone, and AY + AFB₁. The cows in the aflatoxin treatment group received AFB₁ from d 1 to 6, while the SC biomass was administered with the AFB₁ bolus from d 4 to 6. Aflatoxin B₁ or SC-based products did not affect milk production or milk composition during the experimental period. Aflatoxin M₁ was detected in the milk from all aflatoxin treatment group cows, reaching maximum levels at d 3 and varying from 0.52 ± 0.03 to 1.00 ± 0.04 µg/L. At end of the treatment period, CW, AY, DY, and BY removed 78%, 89%, 45%, and 50% of AFM₁ from the milk, respectively, based on the highest level found on d 3. Results indicate a potential application of industrial fermentation by-products, especially CW and AY, as a feed additive in the diets of dairy cows to reduce the excretion of AFM₁ in milk.

Key words: aflatoxin M₁, residues, dairy, yeast-based product, mass spectrometry

INTRODUCTION

Aflatoxin B₁ (AFB₁) is a toxic secondary metabolite produced by fungi of the genus *Aspergillus*, particularly *A. flavus*, *A. parasiticus*, and *A. nomius*, that grow naturally in several agricultural commodities and hence contaminate food or feed, leading to human health risks and economic losses in animal production (Oliveira and Corassin, 2014). Exposure to dietary levels of AFB₁ is considered a serious public health problem worldwide because of its carcinogenic, mutagenic, teratogenic, immunosuppressive, and hepatotoxic effects (Oliveira and Corassin, 2014; Oliveira et al., 2014). Aflatoxin B₁ ingested by lactating animals is biotransformed in the liver to a monohydroxylated metabolite, aflatoxin M₁ (AFM₁), which is subsequently excreted in the milk (Firmin et al., 2011). The toxic and carcinogenic effects of AFM₁ have also been extensively demonstrated in laboratory species (Riley and Pestka, 2005). The International Agency for Research on Cancer (IARC, 2002) has classified both AFB₁ and AFM₁ as agents belonging to Group 1, carcinogenic for humans.

In dairy cows, the main manifestations of aflatoxicosis include a decrease in feed consumption, reduced milk production, and weight loss. In addition, the liver may display signs of fatty degeneration, fibrosis, and tumors (Gonçalves et al., 2015a). However, a few studies have reported contradictory results for the effects of AFB₁ on milk yield and composition. González et al. (2004) observed that 76.4 µg/kg of aflatoxins in the diet (43.5 µg of AFB₁) of dairy cows for 6 mo was sufficient to reduce the milk yield. Similar findings were reported by Queiroz et al. (2012), with cows receiving 75 µg of AFB₁/kg during a period of 12 d having lower levels of milk fat and protein. In contrast, Kutz et al. (2009) found that dairy cows fed 112 µg of AFB₁/kg of DM for 28 d did not have any alteration in milk fat and protein concentration. Aflatoxin B₁-contaminated

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¹Corresponding author: carloscorassin@usp.br

diets may cause lower DMI, decreased milk production, BW loss, laminitis, decreased immunity, and signs of fatty degeneration, necrosis, and carcinomas in the liver (Gonçalves et al., 2015a).

The most important outcome of the exposure of dairy cows to dietary levels of AFB₁, even at subtoxic concentrations, is the AFM₁ excretion in milk. Detectable levels of AFM₁ in milk are usually found in the milking following AFB₁ ingestion (Diaz et al., 2004). The rate of AFB₁ transfer from feed to AFM₁ in milk in dairy cows is around 1 to 2% ingested through contaminated feed, although these values may vary up to 6% of the AFB₁ intake (EFSA, 2004). However, Friedman et al. (2010) observed that some cows can have exceptionally high excretion (with levels at 155% above the mean), and they proposed that such cows may occur naturally in a particular genetic pool. Factors such as level of AFB₁ ingested, milk production, stage of lactation, mammary gland health status, and individual sensitivity to aflatoxins also affect the amount of AFM₁ excreted (Xiong et al., 2015). Considering the toxicity of AFM₁, several countries have determined maximum residue levels (MRL) allowed in milk, as well as a tolerance limit for AFB₁ in feed for dairy cows. In the United States and other countries, including Brazil, the MRL for AFM₁ is 0.5 µg/L, which is 10 times higher than the limited adopted by the European Commission (0.05 µg/L) in regulation number 1881/2006 (Oliveira and Corassin, 2014).

Although good agricultural practices and appropriate storage conditions are considered the best way to prevent the exposure of dairy cows to aflatoxin-contaminated feed, several methods have been proposed to reduce the carry-over of AFB₁ from feed to AFM₁ in milk, including physical, chemical, or biological treatments for elimination, inactivation, or reduction of the compound's bioavailability in feed (Queiroz et al., 2012; Oliveira et al., 2014). Among biological approaches, the use of microorganisms that can bind to aflatoxins provides an interesting alternative for reducing AFB₁ bioavailability in cattle feed by enterosorption, thus decreasing or preventing the excretion of AFM₁ (Corassin et al., 2013). In this context, *Saccharomyces cerevisiae* (SC) cells and related products have been widely studied (Corassin et al., 2013; Bovo et al., 2015; Ismail et al., 2016, 2017), taking into account their high availability as yeast biomass residues resulting from the alcoholic fermentation industry. In Brazil, several SC-based products derived from sugarcane fermentation are available in the market, including cell wall (CW), a prebiotic additive composed of glucans and mannan-oligosaccharides; dried yeast (DY), which is the yeast inactivated and submitted to a drying process; and the autolyzed yeast (AY) obtained by a process that

boosts the rupturing of cells by endogenous enzymes. Additionally, SC-based biomass from the Brazilian brewery industry is economically important, especially the partially dehydrated brewery yeast (BY). Bovo et al. (2015) observed that inclusion of 1% of beer fermentation residue in the diet of broilers fed 2.0 mg/kg of AFB₁ reduced the severity of histological changes in the liver and kidney. However, to our knowledge, no previous studies have been published regarding the ability of the previously mentioned SC-based products to prevent the adverse effects of AFB₁ in dairy cows or to reduce AFM₁ excretion in milk. The aim of the present study was to evaluate the effect of different sources of SC-based biomass obtained from the Brazilian sugarcane and beer industry on milk production, milk composition, and AFM₁ excretion in milk from dairy cows receiving AFB₁.

MATERIALS AND METHODS

Experimental Design, Animals, and Diets

Ethics approval was obtained through the ethics committee on the use of animals of the College of Animal Science and Food Engineering (University of São Paulo, Pirassununga, Brazil) before commencement of the study. Twenty lactating multiparous Holstein cows in late lactation (DIM = 216.5 ± 97.5, milk yield = 18.2 ± 1.2 L) were assigned to this trial. Cows were housed in a freestall barn equipped with fans and sprinklers that were automatically triggered when the ambient temperature exceeded 28°C. Cows were milked in parlors twice a day and the milk yield was measured on d 1, 4, and 7 of the experiment by the management software of the milking system. The diet was formulated according to National Research Council (NRC, 2001) for Holsteins cows (550 ± 50 kg of BW), BCS of 3.5 ± 0.5, producing 15 to 20 kg of milk per day. Cows were fed a TMR composed of corn silage, grain concentrate, vitamins, and minerals. Animals had access to clean and fresh water at all times. Additionally, duplicate samples (250 g) of the TMR diet were collected and analyzed for determination of endogenous aflatoxins (AFB₁, AFB₂, AFG₁, and AFG₂) by HPLC, as described by Ledoux and Rottinghaus (1999). Only AFB₁ was detected in the diets at low, nonrelevant levels for the experiment (0.22 ± 0.02 µg/kg).

A completely randomized design was used with 2 dairy cows assigned to each of 10 treatments for 10 d, as follows: (A) negative controls (no SC-based product or AFB₁ administered to animals); (B) positive controls (AFB₁ alone); (C) CW alone; (D) CW and AFB₁; (E) AY alone; (F) AY and AFB₁; (G) DY alone; (H) DY and AFB₁; (I) BY alone; and (J) BY and AFB₁. Afla-

toxin B₁ used in the experiment was produced from *A. parasiticus* (NRRL-2999), according to Shotwell et al. (1966). The quantification of total aflatoxins was made by HPLC, as described by Ledoux and Rottinghaus (1999). Aflatoxin B₁ was the major aflatoxin compound in the culture material (124.21 mg/kg), while the concentrations of aflatoxins B₂, G₁ and G₂ were 0.32, 39.51, and 12.11 mg/kg, respectively. The aflatoxin culture material was administered orally to cows from treatments B (positive control), D, F, H, and J, using 2 capsules containing 120 µg of AFB₁ each, immediately after the morning and afternoon milking (total daily intake: 480 µg AFB₁), for 6 consecutive days (starting on d 1 of the experiment).

The SC-based products used in the experiment were commercially available and were kindly donated by the company ICC Brazil (São Paulo, SP, Brazil). The SC-based products (20.0 g/d, containing 8.0×10^{10} cells-equivalent per gram) were administered orally in capsules to cows from all treatments, except A and B (negative and positive control, respectively), immediately after the morning and afternoon milking, for 7 consecutive days, starting on d 4 of the experiment. The amount of SC-based biomass ingested was based on previous in vitro results showing at least 90% binding by 2×10^{10} cells to 5.0 µg of AFB₁, as described by (Gonçalves et al., 2015b). In summary, the experiment was conducted for 10 consecutive days, comprising 3 initial days of dairy cows exposure to AFB₁ alone, followed by 3 d of simultaneous exposure to AFB₁ and administration of SC-based products, and finally 4 d with SC-based products alone, as well as negative and positive controls.

Milk Collection and Determination of AFM₁

According to the recommendations of the International Dairy Federation (IDF, 1996), milk samples (approximately 100 mL) were collected from each cow at each of the 2 daily milkings (morning and evening). These samples were mixed thoroughly in a ratio according to the milk yield from each milking and then divided into 2 equal subsamples of 100 mL each. One subsample taken on d 1, 4, and 7 of the experiment was immediately analyzed for milk fat and protein using a Lactoscan MCC (Milkotronic, Nova Zagora, Bulgaria). The other subsamples obtained daily during the 10 d of the experiment were prepared as described by Xiong et al. (2013) and submitted for quantification of AFM₁ using a Waters Acquity I-Class ultra-performance liquid chromatograph (UPLC) system equipped with a BEH C₁₈ column (2.1 mm × 50 mm, 1.7 µm) and coupled to a Xevo TQ-S mass spectrometer (Waters Corp., Milford,

MA). The column was kept at 40°C during analyses, and samples were maintained at 10°C. Ten microliters of sample extracts and standards were injected. Gradient elution was employed with mobile phase composed for water (eluent A) and acetonitrile (eluent B), both containing 0.05% of aqueous ammonia. After an initial period of 0.2 min at 90% A, the percentage of B was linearly raised by 60% over 1.1 min (1.3 min). Then, eluent B was increased to 90% over 0.05 min, followed by a hold time of 0.25 min (1.6 min). Following this, the percentage of B was reduced to 10% over 0.1 min (1.7 min), and the column re-equilibrated to initial conditions for 0.8 min. Total chromatographic run time was 2.5 min and the mobile phase flow rate was maintained at 0.6 mL/min. The mass spectrometer was operated in multi-reaction monitoring (MRM) mode using electrospray ionization in positive ion mode, with a capillary voltage of 0.75 kV, a source temperature of 150°C, and a desolvation temperature of 650°C. Desolvation gas flow and cone gas flow were maintained at 500 and 150 L/h, respectively. Cone voltage, collision energy, and MRM transitions (major precursor ion > fragment ion) were automatically optimized for each compound individually using Intellistart program of Acquity UPLC console. The most intense transition was set for quantification and the second most intense was used as confirmatory transition. Quantification and confirmatory MRM transitions were m/z 329 > 273 and 329 > 229, respectively. Data collection and processing were performed using software MassLynx version 4.1. The performance of the analytical method for AFM₁ in milk samples has been presented elsewhere (Jager et al., 2013), describing limits of detection and quantification of 0.0025 and 0.0080 µg/L, respectively.

Aflatoxin M₁ levels found in milk (µg/L) on each sampling day were multiplied by the milk volume produced (L/d) to obtain the total amount of AFM₁ excreted daily into milk (µg/d). This value was used to calculate the percentage of carry-over of the ingested AFB₁ excreted daily into milk as AFM₁:

$$\text{Carry-over (\%)} = \frac{\text{excretion of [total AFM}_1 \text{ excreted (}\mu\text{g/d)]}}{\text{AFB}_1 \text{ ingested (}\mu\text{g/d)}} \times 100.$$

Statistical Analysis

Data were analyzed as a completely randomized design by ANOVA using the General Linear Model procedure of SAS (SAS Institute, 2004). The means for treatments showing significant differences in the ANOVA were compared using Tukey test. Statistical significance was accepted at $P < 0.05$.

Table 1. Milk production and fat and protein contents in milk from dairy cows orally dosed with aflatoxin B₁ (AFB₁), *Saccharomyces cerevisiae* (SC)-based products, or both on d 1, 4, and 7 of the experiment^{1,2}

Treatment	SC-based product ³	AFB ₁ (µg/d)	Milk production (kg/d)			Fat (%)			Protein (%)		
			d 1	d 4	d 7	d 1	d 4	d 7	d 1	d 4	d 7
A ⁴	—	—	18.0	17.5	19.0	3.93	3.84	3.83	3.01	3.06	2.99
B ⁵	—	480	17.0	17.0	16.5	3.94	3.86	3.88	2.85	2.96	2.82
C	CW	—	18.5	19.0	19.0	3.88	4.04	3.92	3.03	3.00	2.94
D	CW	480	16.5	17.5	19.0	3.72	3.97	3.88	3.08	3.07	3.00
E	AY	—	21.0	21.0	20.5	3.92	3.83	3.81	2.83	2.79	2.81
F	AY	480	17.5	19.0	16.5	3.83	3.92	3.94	2.78	2.82	2.75
G	DY	—	19.0	18.0	18.5	3.84	3.91	3.78	3.03	2.97	3.01
H	DY	480	17.0	18.0	17.0	3.52	3.73	3.68	3.10	3.05	3.08
I	BY	—	19.5	21.0	19.5	3.85	4.01	3.93	3.01	2.94	2.98
J	BY	480	19.0	18.5	17.5	3.94	3.88	4.01	2.98	2.91	2.82
SEM			0.32	0.30	0.31	0.03	0.02	0.03	0.02	0.03	0.02

¹No significant difference ($P > 0.05$) was found for means in the same column using the Tukey's test.

²Days 1, 4, and 7 are the first day of the treatment period, first day after starting administration of SC-based products, and first day after withdrawal of the toxin, respectively.

³CW = cell wall yeast from sugarcane industry; AY = autolyzed yeast from sugarcane industry; DY = dried yeast from sugarcane industry; BY = partially dehydrated yeast from brewery industry.

⁴Negative control.

⁵Positive control.

RESULTS AND DISCUSSION

No changes were observed in the general health status of the cows during the experimental period. Table 1 presents the milk production and fat and protein contents in milk on d 1, 4, and 7 of the experiment. No differences ($P > 0.05$) were observed in the parameters evaluated, demonstrating that daily oral doses of AFB₁ (480 µg/d) or SC-based products under conditions of the present experiment had no negative effects on the lactation performance of dairy cows. These results are in agreement with those reported in previous works. Stroud et al. (2006) did not report any alteration in milk yield after feeding cows with 170 µg of AFB₁/kg of DMI over 11 d, although the toxin decreased the feed intake almost 1.5 kg/d compared to the intake of cows fed AFB₁-free diets. In the trial conducted by Kutz et al. (2009), dairy cows ingesting 112.2 µg of AFB₁/kg of DM also had no change in the milk yield or milk composition. In other studies testing SC-based products in dairy cows (Firmin et al., 2011; Mojtahedi et al., 2013), the results also indicated no AFB₁ effects on milk production or composition, similarly to the present experiment. However, Guthrie and Bedell (1979) reported that milk yield was significantly reduced after feeding dairy cows 120 µg of aflatoxins/kg of BW. Veldman et al. (1992) also reported a decrease in milk production of Holstein cows treated with direct administration of 13 µg of AFB₁/d in the rumen (through fistulae) for 7 d. Gonzalez et al. (2004) found that levels as low as 43.5 µg of AFB₁ were sufficient to reduce average milk production by 3 L/d through an extended 6-mo period

of treatment. Negative effects of AFB₁ on milk fat or protein concentrations have been described at lower levels of exposure (Queiroz et al., 2012). The reduction in milk protein concentration may be a consequence of the inhibition of protein synthesis by AFB₁ (Garvican et al., 1973). Thus, taken together, the conflicting data reported in the literature and those described in the present study indicate a high variability in susceptibility of dairy cows to the toxic effects of AFB₁, which may vary according to the breed, milk productivity, length of exposure to the toxin, method of administration, and individual sensitivity, among other factors.

Aflatoxin M₁ concentrations in milk samples from cows not subjected to oral doses of AFB₁ (treatments A, C, E, G, and I) were below the quantification limit (0.008 µg/L), which was expected based on the low levels of AFB₁ in the experimental feed. The levels of AFM₁ found in milk samples from dairy cows fed AFB₁ (treatments B, D, F, H, and J) are summarized in Table 2. As expected, AFM₁ was first detected in milk on the following day after administration of AFB₁ capsules, hence confirming that AFB₁ is promptly absorbed by the gastrointestinal tract and rapidly biotransformed and excreted into milk as AFM₁ (Frobish et al., 1986; Kuilman et al., 2000; Battacone et al., 2003, 2009). All SC-based products investigated were able to reduce the AFM₁ transfer in milk, although CW and AY showed higher capacity ($P < 0.05$) on d 6 of experiment compared with DY and BY. Milk from positive controls had AFM₁ levels higher than the Brazilian regulations (0.5 µg/L) starting on d 3 and continued to increase until reaching 1.1 µg/L on d 6 (last day of aflatoxin

Table 2. Levels of aflatoxin M₁ (AFM₁; µg/L) in milk from dairy cows orally dosed with aflatoxin B₁ (480 µg/d) and *Saccharomyces cerevisiae* (SC)-based products during 10 consecutive days¹

Treatment	SC-based product ²	Day of trial									
		1	2	3	4	5	6	7	8	9	10
B ³	—	0.24	0.42 ^b	0.65 ^b	0.77 ^a	0.93 ^a	1.10 ^a	0.65 ^a	0.37 ^a	0.20 ^a	ND ⁴
D	CW	0.23	0.39 ^b	0.58 ^b	0.41 ^c	0.25 ^c	0.13 ^d	0.09 ^c	0.07 ^c	ND ^c	ND
F	AY	0.19	0.55 ^a	1.00 ^a	0.74 ^a	0.53 ^b	0.11 ^d	0.08 ^c	0.06 ^c	ND ^c	ND
H	DY	0.21	0.50 ^a	0.70 ^b	0.55 ^b	0.47 ^b	0.39 ^b	0.25 ^b	0.16 ^b	0.11 ^b	ND
J	BY	0.18	0.40 ^b	0.52 ^c	0.43 ^c	0.30 ^c	0.26 ^c	0.19 ^b	0.11 ^b	0.09 ^b	ND
SEM		0.006	0.015	0.042	0.037	0.060	0.091	0.052	0.028	0.014	0

^{a-d}In the same column, means followed by different letters differ significantly ($P < 0.05$).

¹Days 1–3, 4–6, and 7–10 comprise the periods of initial treatment (only aflatoxin B₁), simultaneous administration of aflatoxin B₁ and SC-based products, and administration of SC-based products only (withdrawal of the toxin), respectively.

²CW = cell wall yeast from sugarcane industry; AY = autolyzed yeast from sugarcane industry; DY = dried yeast from sugarcane industry; BY = partially dehydrated yeast from brewery industry.

³Positive control.

⁴ND = not detected (limit of quantification: 0.008 µg/L).

treatment period). Therefore, no plateau in AFM₁ concentrations was observed in the milk from positive control cows after 6 d of daily ingestion of 480 µg AFB₁, possibly because the time elapsed was not enough for reaching the plateau. Previous studies indicate a high variability in the time required for reaching maximum AFM₁ levels in milk after continuous administration of the same amount of AFB₁ to lactating animals, with a plateau achieved by 24 h (Frobish et al., 1986), 3 d (Diaz et al., 2004), and 7 d (Masoero et al., 2007) for dairy cows and 9 d for sheep (Battacone et al., 2003). However, milk from CW and BY treatments only had levels of AFM₁ higher than the tolerance limit on d 3, whereas AY and DY milks had higher levels on d 3 and d 3 to 4, respectively.

Table 3 presents the percentages of carry-over of AFB₁ into AFM₁ in milk from dairy cows dosed with SC-based products for 10 consecutive days. The maxi-

mum carry-over value (3.95%) was found in cows from AY treatment on d 3 of the experimental period. The lowest value (0.2%) was obtained on d 7 to 10 for AY treatment. The CW and AY treatments had the lowest carry-over values ($P < 0.05$), compared with the other treatments, starting on d 4 and 5, respectively. The DY and BY treatments also had milk with lower carry-over percentages compared with the positive controls. These values are in agreement with those mentioned by the European Food Safety Authority (EFSA, 2004) as predictable for carry-over rates in dairy cows (1–6%). However, the carry-over values obtained in the present study were slightly higher than those described in previous studies with dairy cows (Applebaum et al., 1982; Xiong et al., 2015) and sheep (Battacone et al., 2005, 2009), hence highlighting the existence of individual variations in the AFM₁ excretion among different animals.

Table 3. Percentage carry-over¹ of aflatoxin B₁ (480 µg/d, orally dosed) into aflatoxin M₁ in milk from dairy cows dosed with *Saccharomyces cerevisiae* (SC)-based products during 10 consecutive days²

Treatment	SC-based product ³	Day of trial									
		1	2	3	4	5	6	7	8	9	10
B ⁴	—	0.83 ^a	1.57 ^b	2.29 ^c	2.86 ^a	3.27 ^a	3.89 ^a	2.16 ^a	1.31 ^a	0.66 ^a	0
D	CW	0.75 ^a	1.30 ^c	2.06 ^d	1.44 ^c	0.93 ^c	0.51 ^d	0.32 ^d	0.26 ^d	0 ^c	0
F	AY	0.66 ^b	1.82 ^a	3.95 ^a	2.91 ^a	1.87 ^b	0.37 ^e	0.25 ^d	0.20 ^d	0 ^c	0
H	DY	0.72 ^a	1.76 ^a	2.62 ^b	1.95 ^b	1.56 ^b	1.36 ^b	0.90 ^b	0.51 ^b	0.36 ^b	0
J	BY	0.69 ^b	1.50 ^b	2.04 ^d	1.80 ^b	1.20 ^c	0.99 ^c	0.67 ^c	0.39 ^c	0.32 ^b	0
SEM		0.015	0.047	0.178	0.147	0.204	0.321	0.173	0.101	0.030	0

^{a-e}In the same column, means followed by different letters differ significantly ($P < 0.05$).

¹Ratio between the total amounts of aflatoxin M₁ excreted in the milk produced daily and the daily intake of aflatoxin B₁ (480 µg).

²Days 1–3, 4–6, and 7–10 comprise the periods of initial treatment (only aflatoxin B₁), simultaneous administration of aflatoxin B₁ and SC-based products, and administration of SC-based products only (withdrawal of the toxin), respectively.

³CW = cell wall yeast from sugarcane industry; AY = autolyzed yeast from sugarcane industry; DY = dried yeast from sugarcane industry; BY = partially dehydrated yeast from brewery industry.

⁴Positive control.

Based on the highest level found in milk on d 3, the relative percentages of AFM₁ levels in milk from dairy cows receiving AFB₁ and SC-based products are presented in Figure 1. On d 4, CW reduced AFM₁ levels in milk at concentrations that represent 69.9% of the highest level found the day before (d 3). The decrease continued on the following days, until reaching 22.5% of the highest AFM₁ level on d 6 of simultaneous administration of AFB₁ and CW. Similar results were observed for AY, although the protective effect against AFM₁ excretion in milk was higher than CW on d 6, reaching only 11% of the highest level found on d 3. The DY and BY products also decreased the AFM₁ levels during treatment period with AFB₁ (d 4–6), but they were less effective than the CW or AY products because the percentages of AFM₁ in milk varied from almost 50 to 55% of the highest levels in milk. Hence, CW, AY, DY, and BY removed 78, 89, 45, and 50% of the AFM₁ from the milk, respectively, based on the highest level found on d 3 of AFB₁ administration. The results for DY and BY are in agreement with the data reported by Diaz et al.

(2004), who used yeast cell extract at 0.05% in the diet of dairy cows containing 55 µg of AFB₁/kg and found a reduction of 59% of AFM₁ levels in milk. In the present study, the removal percentages of AFM₁ by CW and AY were much higher than previous reports using yeast cell wall or clay adsorbents (40–60%). Although the binding mechanisms between yeast cell wall and AFB₁ are not completely elucidated, our results suggest that CW and AY have a higher number of binding sites for AFB₁ or higher stability of the AFB₁–product complex in the gastrointestinal tract of ruminants compared with other adsorbent products. Coherently, the same SC-based products evaluated in the present study were previously tested in vitro using AFB₁ dissolved in PBS (0.5 µg/mL), and the reported binding percentages of CW and AY were 86.1 and 90.4% (Gonçalves et al., 2015b). However, Kutz et al. (2009) observed only a 4% reduction in the AFM₁ concentration in milk from dairy cows fed diets containing 100 µg of AFB₁/kg and yeast cells at 0.5% of DM. No effects of SC-based products on milk AFM₁ levels were also reported by Battacone et al.

Figure 1. Relative percentages of aflatoxin M₁ levels in milk from dairy cows orally dosed with aflatoxin B₁ (480 µg/d) and *Saccharomyces cerevisiae* (SC)-based products during 10 d. Error bars indicate SD. All values were calculated based on 100% being the highest level found in milk after a previous 3-d exposure of cows to aflatoxin B₁ alone in each treatment (on d 3). Days 4 to 6 and 7 to 10 comprised the periods of simultaneous administration of aflatoxin B₁ and SC-based products and SC-based products only (i.e., withdrawal of the toxin), respectively. CW = cell wall yeast from sugarcane industry; AY = autolyzed yeast from sugarcane industry; DY = dried yeast from sugarcane industry; BY = partially dehydrated yeast from brewery industry.

(2009) and Firmin et al. (2011), after adding yeast cell wall and dried yeast in diets containing aflatoxin (60 µg/kg, naturally contaminated). According to Xiong et al. (2015), the variability of data reported on the protective effect of yeast against AFM₁ excretion in milk in different studies may be related to differences in the animal species used in the experiments and in the composition of products tested. Ruminants have a complex metabolism and many factors can influence the adsorption capacity of yeast in the gastrointestinal tract, among them one of the most important is the composition of rumen fluid. Moreover, the mechanism for the protective effects of SC-based products against AFB₁ in chickens seems to involve the sequestration of the toxin in the gastrointestinal tract and chemisorption to yeast cells with its subsequent elimination in feces (Bovo et al., 2015). However, the adsorption of AFB₁ by SC-based products is not a completely linear phenomenon and may vary according to several factors, especially the amount of β-D-glucans and conformation of the yeast cell wall, which differs among yeast strains (Jouany and Diaz, 2005), hence contributing to the differences observed in the mentioned studies. In the present study, the fact that all the SC-based products orally administered to lactating animals were able to reduce AFM₁ excretion into milk indicates a potential for incorporating these products in the feed for dairy cows.

CONCLUSIONS

The continuous oral administration of 480 µg of AFB₁ for 6 d along with 4 different SC-based products to dairy cows did not affect milk yield or the concentration of milk fat or protein. Although the SC-based biomass tested were able to reduce the concentration of AFM₁ in milk, the CW and AY products presented higher efficiency, removing 78 and 89% of the AFM₁ from the milk, respectively, based on the highest level found on d 3 of AFB₁ administration. Results indicate a potential application of industrial fermentation by-products containing yeast cells, especially CW and AY, in reducing the bioavailability of AFB₁ in the diets of dairy cows and the excretion of AFM₁ in milk.

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